

Chemical synthesis of oligonucleotides using mild reagents aiming to tag synthesis on-chip for single cell analysis with positional information

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We are trying to develop an analytical method for genome expression in single cell with positional information in tissues and cell network, using short DNA namely address tag containing the positional information. Here, we are focusing on the combinatorial syntheses of address tag based oligonucleotides on chip. PDMS based microfluidic chip was developed for on-chip syntheses of oligonucleotides. Phosphoramidite based DNA synthesis cycle consists of a series of steps including deblocking, activation, capping and oxidation, while washing step was performed between each successive step. Fig 1 shows the experimental setup for the synthesis of on-chip oligonucleotide. One nucleotide per cycle was added to the support. Although conventional DNA synthesis scheme was fully developed and optimized for high purity and yield, however, conventional deprotecting reagent (3% TCA in DCM) was not compatible for Polydimethylsiloxane (PDMS) micro channels as they were damaged and clogged during DCM exposure. Hence in this work, another deprotecting reagent (10% TFA in acetonitrile) was used as a mild deblocking reagent for the DNA synthesis using PDMS chip.

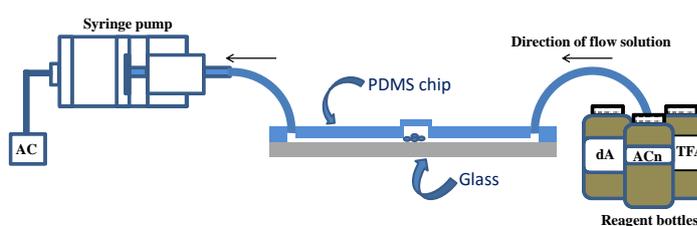


Figure 1. Experimental setup for solid phase oligonucleotide synthesis on PDMS chip

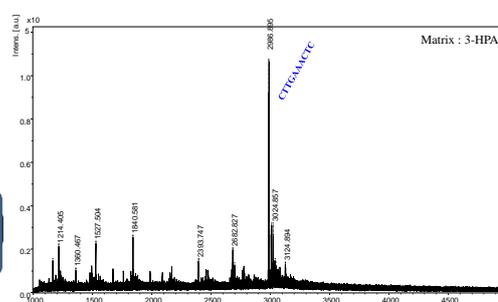


Figure 2. MALDI-TOF analysis of 10-mer oligonucleotide synthesized using mild reagents

We confirmed the sequence of synthesized oligonucleotide by MALDI-TOF mass spectrometry and gel electrophoresis. Fig. 2 shows the MALDI-TOF spectra of on-chip synthesized 10-mer oligonucleotide.

References:

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